BSI Cambridge Immunology Symposium:
Pushing boundaries in immunology
Friday 14 April 2023
Queens' College, Cambridge

FRIDAY 14 APRIL

08:30	Registration and coffee
09:15	**Welcome** Clare Bryant, University of Cambridge, UK
	SESSION 1 Chair: Felix Randow, University of Cambridge, UK
09:15	**Immune response to DNA - from human to bacteria and back** James Chen, UT Southwestern, USA – *invited speaker*
10:00	**Lost in Space: How ageing makes Tfh cells lose their way.** Michelle Linterman, Babraham Institute, UK – *invited speaker*
10:45	Refreshment break, posters and meet the exhibitors
	SESSION 2 Chair: Virginia Pedicord, University of Cambridge, UK
11:15	**Memory T Cell Surveillance and Longevity** David Masopust, University of Minnesota, USA – *invited speaker*
12:00	**Systemic autoimmunity: where we are and what the future may hold…** Carola Vinuesa, The Francis Crick 1nstitute, UK – *invited speaker*
12:45	Lunch, posters and meet the exhibitors
	SESSION 3 Chair: Brian Ferguson, University of Cambridge, UK
14:30	**Pushing the boundaries of central nervous system immunity** Menna Clatworthy, University of Cambridge, UK– *invited speaker*

15:15	**Why so many ways to Die: Cell death drives inflammation** Vishva Dixit, Genentech, USA– *invited speaker*
16:00	Refreshment break, posters and meet the exhibitors
	SESSION 4 Chair: Clare Bryant, University of Cambridge, UK
16:30	**Dark kinases and their role in autoimmunity and anti-tumor immunity.** Vijay Kuchroo, Harvard Medical School, USA – *invited speaker*
17:15	Poster awards and thanks
17:45	Drinks reception
18:30	Dinner (for those who have registered to attend)

Poster presentations

P.01 MHC-II expression by glia cells in the CNS after lysolecithin-induced demyelination

Kristina Ulicna, Newcastle University, UK, Wellcome-Wolfson Institute of Experimental Medicine, Queen´s University Belfast, UK

P.02 B cells from older bodies are not intrinsically defective in responding to stimulation and differentiating into antibody-secreting cells

Jia Le Lee, Babraham Institute, UK

P.03 Clostridium septicum alpha-toxin activates the NLRP3 inflammasome by engaging glycosylphosphatidylinositol (GPI)-anchored proteins

Weidong Jing, Australian National University, Australia

P.04 MAIT cells activate dendritic cells to promote Tfh cell differentiation and induce humoral immunity

Theresa Pankhurst, Victoria University of Wellington, NZ, Malaghan Institute of Medical Research, NZ

P.05 High levels of age-associated B cells predict impaired humoral immunity after COVID-19 vaccination

Juan Carlos Yam-Puc, Medical Research Council Toxicology Unit, School of Biological Sciences, University of Cambridge, UK

P.06 Post-transcriptional regulation by ZFP36L1 establishes the immunodominant CD8 T cell response

Georg Petkau, Immunology Programme The Babraham Institute, Babraham Research Campus, UK

P.07 Are fibroblasts Immune Cell Filters in Peripheral Tissues?

Zhi Yi Wong, Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK

P.08 Full-length transcriptome sequencing reveals mechanisms underpinning the positive selection of B cells in germinal centers

Ozge Gizlenci, Immunology Programme, The Babraham Institute, University of Cambridge, UK

P.09 The effect of platinum chemotherapy on natural killer cells in murine High Grade Serous Ovarian Cancer cell lines in vitro and in vivo

Christabel Boyles, CRUK CI, University of Cambridge, UK

P.10 Immune and stromal crosstalk in the early stages of ovarian cancer metastasis: a role for type-2 innate lymphoid cells?

Moreno-Vicente J., Yip, Cancer Research UK Cambridge Institute, University of Cambridge, UK

P.11 Dynamic mitochondrial transcription and translation in B cells control germinal centre entry and lymphomagenesis

Yavuz F Yazicioglu, Kennedy Institute of Rheumatology, University of Oxford, UK

P.12 BACH2 regulates thymic negative selection through restraining single-positive thymocyte activation

Alexander C. Evans, Department of Pathology, University of Cambridge, UK

P.13 Giving Toll Like Receptor 4 Signalling an Angelic Glow

Gabrielle McClymont, University of Cambridge, UK

P.14 Exploring the synovial tissue-homing properties of human mononuclear phagocytes in active treatment naïve psoriatic arthritis.

Joseph Hutton, Department of Pathology, University of Cambridge, UK, Rheumatology Research Unit, Cambridge University Hospitals NHS Foundation Trust, UK, Department of Medicine, University of Cambridge, UK

P.15 Early infection response of the first-trimester placenta at single cell resolution

Elias R. Ruiz-Morales, Wellcome Sanger Institute, Cambridge, UK,

P.16 Establishing tools to study the microbicidal capacity of human placental macrophages across gestation

Nagisa Yoshida[1], Department of Pathology, University of Cambridge, UK, Centre for Trophoblast Research, University of Cambridge, UK

P.17 Multiviral Quartet Nanocages Elicit Broad Anti-Coronavirus Responses for Proactive Vaccinology

Rory Hills, Department of Biochemistry, University of Oxford, UK, Department of Pharmacology, University of Cambridge, UK

P.18 Investigating the roles of ZFP36 and ZFP36L1 RNA binding proteins in the regulation of CD8+ T cell functions

Marian Jones Evans, The Babraham Institute, UK

P.19 Effects of the commensal microbiota on T cell-mediated intestinal inflammation in response to immunotherapy.

Wangmingyu Xia, Cambridge Institute of Therapeutic Immunology & Infectious Disease, University of Cambridge, UK

P.20 Identification of novel in vivo regulators of CD8 T cell persistence

Holly Robertson, University of Cambridge, UK

P.21 Diminished Anti-HCMV T cell Functionality in the elderly may be ameliorated by inhibition of the PD-1: PD-L1 axis

Sarah E. Jackson, Department of Medicine, Cambridge Institute of Therapeutic Immunology and Infectious Disease, University of Cambridge School of Clinical Medicine, UK,

P.22 Antigen drainage to the B cell follicle of the lymph node is impaired in aged mice

Xin Ge, Immunology Programme, Babraham Institute, UK

P.23 Building a description of the ex vivo immunometabolism of Leishmania panamensis-infected macrophages from RNA-seq data

Julieth Murillo, University of Cambridge, UK, Javeriana University, Columbia

P.24 Development of a rapid screening platform for mRNA vaccines

Maria Rust, University of Cambridge, UK

P.25 PI3Kδ activity in T or B cells: Different drivers of B cell lymphoma in the context of deregulated BCL6

Julius C. Baeck, Department of Pathology, University of Cambridge, UK,

P.26 Integrin αvβ3 potentiates TH2 cell immunity

Aydan C. H. Szeto, MRC Laboratory of Molecular Biology, UK

P.27 Clustered invasion triggers cytosolic release of Salmonella Paratyphi A and subsequent cytosolic motility favors evasion of xenophagy

Felix Scharte, MRC Laboratory of Molecular Biology, UK, University of Osnabrück, Germany

P.28 CD14 marks tissue CD8^{+}T-cells instructed by myeloid cells and modulated by LPS

Laura J. Pallett, Division of Infection & Immunity, Institute of Immunity & Transplantation, University College London, UK

The British Society for Immunology like to thank the following sponsors for their support:

Poster abstracts

P.01 MHC-II expression by glia cells in the CNS after lysolecithin-induced demyelination

Kristina Ulicna[1,2], Jessica White[2], Mohammad Mofatteh[2], Denise Fitzgerald[2]

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In multiple sclerosis, myelin sheaths around the axons are damaged during demyelination and oligodendrocytes are not always successful in repairing them, resulting in disease progression. Although there are no treatments for Multiple sclerosis (MS), it was shown that regulatory T cells can drive oligodendrocyte progenitor cell (OPC) differentiation and remyelination. However, very little is known about the mechanisms by which Tregs drive these processes. We identified MHC-II as molecule of interest, as it is involved in the traditional T-cell activation pathways. As MHC-II is upregulated in the CNS by glial cells that are important for myelin regeneration, we hypothesized that MHC-II might be required for efficient OPC differentiation and remyelination; therefore, we aimed to investigate the expression of MHC-II throughout this process. We used an in vivo model of lysolecithin-induced demyelination in WT mice and stained by immunofluorescence for microglia, astrocytes, oligodendrocytes, and MHC-II at four different time points. Based on the data collected, it was observed that microglia (brain macrophages) are the highest expressers of MHC-II at all time points, with a peak at 10-day-post-lesion. On the other hand, astrocytes and oligodendrocytes express low levels of MHC-II, suggesting that it may not be required for efficient myelin regeneration. Understanding the localization of MHC-II expression could help to identify its role during remyelination and whether glia cells functions could be impaired in its absence. Investigating MHC-II expression could also help identify whether Treg immune responses coincide with upregulation, its role in remyelination and the development of potential therapies for MS.

P.02 B cells from older bodies are not intrinsically defective in responding to stimulation and differentiating into antibody-secreting cells

Jia Le Lee[1], Sigrid C. Fra-Bido[1], Alice R. Burton[1], Silvia Innocentin[1], Danika L. Hill[1,2], Michelle A. Linterman[1]

[1]Babraham Institute, UK, [2]Monash University, Australia

Ageing is often associated with a reduction in antibody-secreting cell formation and vaccine-induced antibody titres, thereby limiting vaccine efficacy. Whether the impaired antibody response during ageing is contributed by intrinsic defects in B cells function remains unclear. Here, we show that B cells from older people do not have intrinsic defects in their proliferation and differentiation into antibody-secreting cells in vitro compared to those from the younger donors. However, adoptive transfer of B cells from aged SWHEL mice to young recipient mice showed that differentiation into extrafollicular plasma cells was favoured at the expense of B cells entering the germinal centre (GC) during the early stages of GC formation. Nevertheless,

by the peak of the GC response, GC B cells derived from the donor cells of aged mice had expanded to the same extent as those from the younger donors. This indicates that age-related intrinsic B cell changes might delay the GC response but are not responsible for the impaired antibody-secreting response or smaller peak GC response in ageing. Using the B1-8 adoptive transfer system, we replicated the data showing that B cells from aged B1-8 mice were equally able to mount a GC response, compared to their younger counterparts. Furthermore, GC B cells derived from aged donor mice had no defects in upregulating cMyc. Collectively, we show that B cells from older bodies are not intrinsically defective in responding to stimulation and becoming antibody-secreting cells, implicating B cell-extrinsic factors as the primary cause of age-associated impairment in humoral immunity.

P.03 Clostridium septicum alpha-toxin activates the NLRP3 inflammasome by engaging glycosylphosphatidylinositol (GPI)-anchored proteins

Weidong Jing[1], Jordan Lo Pilato[1], Callum Kay[1], Shouya Feng[1], Daniel Enosi Tuipulotu[1], Anukriti Mathur[1], Cheng Shen[1], Chinh Ngo[1], Anyang Zhao[1], Lisa Miosge[1], Sidra Ali[1], Elizabeth Gardiner[1], Milena Awad[2], Dena Lyras[2], Avril Robertson[3], Nadeem Kaakoush[4], Si Ming Man[1].

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Clostridium species are a group of Gram-positive bacteria that cause human diseases, such as food poisoning, botulism, and tetanus. Here, we analysed ten different Clostridium species and identified that Clostridium septicum, a pathogen that causes sepsis and gas gangrene, activates the mammalian cytosolic inflammasome complex in mice and humans. Mechanistically, we demonstrate that alpha-toxin secreted by C. septicum binds to glycosylphosphatidylinositol (GPI)-anchored proteins on the host plasma membrane, oligomerising and forming a membrane pore that is permissive to efflux of magnesium and potassium ions. Efflux of these cytosolic ions triggers the activation of the innate immune sensor NLRP3, inducing activation of caspase-1 and gasdermin D, secretion of the proinflammatory cytokines interleukin-1beta and interleukin-18, pyroptosis, and plasma membrane rupture via NINJ-1. Furthermore, alpha-toxin of C. septicum induces rapid inflammasome-mediated lethality in mice, and pharmacological inhibition of the NLRP3 inflammasome using MCC950 prevents C. septicum-induced lethality. Overall, our results reveal that cytosolic innate sensing of alpha-toxin is central to recognising C. septicum infection and that therapeutic blockade of the inflammasome pathway may prevent sepsis and death caused by toxin-producing pathogens.

P.04 MAIT cells activate dendritic cells to promote Tfh cell differentiation and induce humoral immunity

Theresa Pankhurst[1,2], Kaitlin Buick[1,2], Ian Hermans[2], Lisa Connor[1]

[1]Victoria University of Wellington, NZ, [2]Malaghan Institute of Medical Research, NZ

Protective immune responses against respiratory pathogens, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus are initiated by the mucosal immune system. However, most licensed vaccines are administered parenterally and are largely ineffective at inducing mucosal immunity. The development of safe and effective mucosal vaccines has been hampered by the lack of a suitable mucosal adjuvant. In this study we explore a novel class of adjuvant that harnesses mucosal-associated invariant T (MAIT) cells.

We show evidence that intranasal immunisation of MAIT cell agonists co-administered with protein, including the receptor-binding domain from SARS-CoV-2 and haemagglutinin from influenza A virus, induced potent humoral immunity and IgA production that provided neutralising protection. MAIT cell adjuvant activity was mediated by CD40L-dependent activation of dendritic cells and subsequent priming of CD4+ T follicular helper cells. In summary, we show that MAIT cells are promising vaccine targets that can be utilised as cellular adjuvants in mucosal vaccines.

P.05 High levels of age-associated B cells predict impaired humoral immunity after COVID-19 vaccination

Juan Carlos Yam-Puc[1†], Zhaleh Hosseini[1†], Emily C. Horner[1†], Pehuén Pereyra Gerber[2,3†], Nonantzin Beristain-Covarrubias[1†], Robert Hughes[1†], Maria Rust[1], Rebecca H. Boston[1], Magda Ali[1], Edward Simmons-Rosello[1], Martin O'Reilly[1], CITIID-NIHR COVID-19 BioResource Collaboration, Sarah Spencer[1], Klaus Warnatz[4,5,6], Rainer Döffinger[7], Christine Parkinson[7], Sara Lear[7], Nicholas J. Matheson[2,3,7,8], James E. D. Thaventhiran[1,7].

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Age-associated B cells (ABCs) accumulate with age and in individuals with a range of immunological conditions, including autoimmune disease, patients with cancer treated with immune checkpoint blockade and patients with inborn errors of immunity, patient groups, particularly at risk from infectious disease. In this study, we sought to determine whether ABCs found in all these environments are similar, and whether they enhance or detract from the response to COVID-19 vaccination. Using single-cell RNA sequencing, we show that ABCs arising from distinct aetiologies have common transcriptional profiles and may be subdivided according to the expression of genes associated with different functions, such as the autoimmune regulator (AIRE). Next, longitudinal profiling of the COVID-19 vaccination response in patients and controls shows that high pre-vaccination ABC frequency correlates with decreased levels of antigen-specific memory B cells, and reduced magnitude and longevity of neutralising capacity against SARS-CoV-2 virus. Preclinical evidence suggests that ABCs might impede humoral vaccine responses by diminishing affinity maturation. However, we found maximal serological neutralising capacity within eight days of vaccination, suggesting alternative mechanisms linking ABC frequency with humoral vaccine deficiency. We discovered that ABCs express the highest levels of the inhibitory FcγRIIB receptor and are the B cell subset best able to bind immune complexes. This could contribute to diminished vaccine responses either directly as a result of inhibitory signalling or indirectly via enhanced clearance of immune complexed-antigen. Expansion of ABCs may therefore serve as a biomarker identifying individuals at risk of suboptimal responses to COVID-19 vaccination.

P.06 Post-transcriptional regulation by ZFP36L1 establishes the immunodominant CD8 T cell response

Georg Petkau, Louise Matheson, Twm J. Mitchell, Martin Turner

Immunology Programme The Babraham Institute, Babraham Research Campus, UK

The T cell effector phase is dominated by few T cells with high affinity TCR for the respective immunodominant antigen. During priming T cells have to process multiple cues in form of antigen, costimulation and cytokines, thus a plethora of T cells with different affinities has to compete for these cues. IL2 has been suggested to be a pivotal pleiotropic molecule which organizes the quantity, quality and clonal composition of T cells contributing to the effector response. How individual T cells compete for and respond to IL-2 and other key cytokines at the molecular level and as a consequence, how this competition shapes population dynamics is still poorly understood. Here we describe how the RNA binding protein ZFP36L1 acts as a sensor of antigen affinity and establishes T cell dominance by promoting the response to IL-2.

P.07 Are fibroblasts Immune Cell Filters in Peripheral Tissues?

Zhi Yi Wong[1], Ilya Korsunsky[2], Matthias Friedrich[1], Mathilde Pohin[1], Mark Coles[1], Christopher Buckley[1]

[1]Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, UK, [2]Division of Rheumatology, Inflammation and Immunity, Brigham and Women's Hospital and Harvard Medical School, USA

In rheumatoid arthritis (RA), like in many chronic immune-mediated diseases, lymphocytes only accumulate in certain regions and around the vasculature. The mechanisms driving lymphocyte compartmentalisation in the peripheral tissues remain obscure, but recent work on organisation and control of germinal centre may hold a clue. It was previously shown that the GGT5-GGG-P2RY8 axis helps confine B cells and T follicular helper cells within germinal centres. We propose that the body uses the same mechanism to compartmentalise leucocytes in the peripheral tissues. Usually, GGT5 expression is limited around the vascular region in health, but it is upregulated and expands into the fibroblast compartments during inflammation. We show that this upregulation is induced by perivascular signals, and TNF kickstarts an autocrine feedback loop that potently stimulates GGT5 expression in fibroblast. In inflamed tissues, GGT5+ fibroblasts and P2RY8+ leucocytes co-localise the same regions, suggesting their interaction. During inflammation, perivascular signals and TNF stimulate GGT5, CXCL1 and CXCL2 expression in fibroblasts. Later when infiltrating monocytes encounter fibroblasts, GGT5 and CCL19 expression are also amplified. As GGT5 is important in regulating the leukotriene pathway that attracts eosinophils and neutrophils, we propose that GGT5+ fibroblasts attract multiple leucocyte subsets into tissues, but specifically use the GGT5-GGG-P2RY8 axis to restrain P2RY8+ leucocytes by restricting their migration. This mechanism prevents them from roaming freely in peripheral tissues by defining a confinement zone to enforce their accumulation in the perivascular space, causing perivascular cuffing.

P.08 Full-length transcriptome sequencing reveals mechanisms underpinning the positive selection of B cells in germinal centers

Ozge Gizlenci[1,2], Louise Matheson[1], Laura Biggins[3], Rebecca Berrens[4], Jingyu Chen[5], Simon Andrews[5], Daniel Hodson[2,5], Martin Turner[1]

[1]Immunology Programme, The Babraham Institute, [2]University of Cambridge, UK, [3]Bioinformatics Group, The Babraham Institute, [4]University of Oxford, UK, [5]Wellcome-MRC Cambridge Stem Cell Institute

Alternative splicing (AS) plays a major role in the differentiation of immune cells during an immune response. 29% of AS genes are specific to the immune system, yet the regulation and function of AS during B cell activation remain largely unknown. Previous studies have linked individual AS events in germinal centre (GC) B cells to B cell malignancies using short-read sequencing; however, defining the complete structures of AS isoforms with short-reads remains a challenge. To overcome this limitation, we developed a long-read sequencing Oxford Nanopore Technologies (ONT) workflow to understand post-transcriptional regulation at both gene and isoform levels in human and mouse GC B cells. Because one of the challenges of ONT is the accurate computational analysis of isoforms, we developed the 'Nexons' pipeline to identify the differentially spliced transcript variants. An in-depth analysis of long-read data with the Nexons revealed the differential regulation of the poison exon (PE) in splicing regulators in GC B cells. During GC reaction, PEs of the splicing factors were preferentially spliced out whereas naïve B cells expressed isoforms carrying PE, leading to nonsense-mediated mRNA decay. Notably, we found this regulation of PE in splicing factors is conserved between human and mouse. Furthermore, we observed AS of the PE of SRSF3 and SRSF7 was induced upon positive selection in mouse GC B cells. Altogether, our findings reveal new insights into AS during B cell activation and positive selection in GC B cells and highlight the SRSF family as important candidates for regulating the GC reaction.

P.09 The effect of platinum chemotherapy on natural killer cells in murine High Grade Serous Ovarian Cancer cell lines in vitro and in vivo

Christabel Boyles[1], Oliver Cast[1], Michael Gill[1], Martin Miller[2], Tim Halim[1]

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Platinum chemotherapeutics are known to induce immune activation in addition to directly inducing cancer cell death. We previously showed that high grade serous ovarian cancer (HGSOC) patient samples are enriched for immune cells, including natural killer (NK) cells, following platinum chemotherapy. Here we investigate how platinum chemotherapy affects NK cell-mediated cytotoxicity in vitro and in vivo. Co-culture assays show that murine HGSOC cell lines are sensitised to NK cell killing following treatment with platinum chemotherapy. This is partially mediated through upregulation of NKG2D ligands on cancer cells. Similarly, NK cell depletion resulted in reduced efficacy of chemotherapy in vivo. Subsequently, we characterised the effect of platinum chemotherapy on the tumour microenvironment in two syngeneic mouse models. A 37-colour flow cytometry immunophenotyping panel was developed to characterise all major immune subsets as well as detailed characterisation of NK and T cell phenotypes. Spatial transcriptomics was undertaken to address the effect of chemotherapy on tumour – immune cell interactions. Collectively, these findings demonstrate the importance of NK cells on mediating the effect of chemotherapy treatment in HGSOC.

Identifying how immune cells may synergise with platinum chemotherapies in HGSOC could guide future immunotherapeutic strategies for this highly lethal

P.10 Immune and stromal crosstalk in the early stages of ovarian cancer metastasis: a role for type-2 innate lymphoid cells?

Moreno-Vicente J., Yip T. CH, Png S., Stockis J., Garcia C., Simpson C., Boyles C., Brenton J., Halim T. YF.

Cancer Research UK Cambridge Institute, University of Cambridge, UK

Metastatic spread of cancer requires a complex reprogramming of the local stromal and immune landscape, which ultimately promotes tumour seeding and growth. Recent work from our group demonstrates that type-2 innate lymphoid cells (ILC2) promote metastases to the lung by suppressing natural killer cell (NK)-mediated anti-tumour activity. Here, we hypothesised that an ILC2-dependent mechanism may similarly promote ovarian cancer metastasis to the omentum, as this organ is particularly enriched in ILC2 and comprises the main site of metastasis. To this end, we deployed high-parameter flow cytometry and engineered mouse models to characterise the early stromal-immune interactions in tumour-bearing omenta. Results showed stromal-cell proliferation and increased IL33 expression early upon tumour injection. Further transcriptional assessment revealed a decrease in inflammatory genes (Il6, Cxcl1) and up-regulation of the myofibroblast marker Acta2. Immune phenotypic analysis showed an increase in omental ILC2 numbers and up-regulation of the activation marker PD-1 early after tumour injection. Likewise, NK numbers were increased, despite a reduction in the expression of effector molecules (IFNγ, GzmB), indicative of NK dysfunction. To test whether stromal-derived IL33 could activate ILC2 and in turn inhibit NK function, we assessed early tumour seeding in IL33- (Il33cit/cit) and ILC2- (Il7rCre/+RoraloxP/loxP) deficient mice. Tumour burden remained unaffected, arguing against a role for stromal-ILC2 interactions in ovarian cancer metastasis. Interestingly, NK depletion did not exacerbate early tumour seeding, which contrasts with the well-characterised anti-metastatic role of NKs in lung metastasis. This highlights that diverse mechanisms might be at play during metastatic seeding to different organs.

P.11 Dynamic mitochondrial transcription and translation in B cells control germinal centre entry and lymphomagenesis

Yavuz F Yazicioglu[1], Eros M Marin[1], Ciaran Sandhu[1], Silvia Galiani[2], Iwan G A Raza[1], Mohammad Ali[3,4], Barbara Kronsteiner[3,4], Ewoud B Compeer[1], Moustafa Attar[1], Susanna J Dunachie[3,4,5,] Michael L Dustin[1], Alexander J Clarke[1*]

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Germinal centre (GC) B cells undergo proliferation at very high rates in a hypoxic microenvironment, but the underlying cellular and bioenergetic processes remain poorly understood. Here, we report that GC B cells have highly dynamic mitochondria, which show significant dependency on mitochondrial transcription and translation mediated by the

transcription factor mitochondrial A (Tfam). Tfam, whilst also necessary for normal B cell development in the bone marrow, is indispensable for the recruitment of activated GC-precursor B cells into the germinal centre reaction. Deletion of Tfam significantly impairs GC formation, function, and output. Loss of Tfam in B cells compromises the actin cytoskeleton and impairs cellular motility of GC B cells in response to chemokines, leading to their spatial disorganisation. Mechanistically, this defect is associated with electron transport chain protein imbalance and mitochondrial reactive oxygen species (mtROS) accumulation, and targeted elimination of mtROS largely rescues the defective mobility in Tfam-deficient B cells. We also demonstrate that B cell lymphoma substantially increases mitochondrial translation, and deletion of Tfam in B cells confers full protection against the development of lymphoma in a c-Myc transgenic mouse model. Finally, we show that pharmacologic inhibition of mitochondrial transcription and translation machinery inhibits the growth of GC-derived human lymphoma cells, disrupts the actin cytoskeleton, and increases mtROS, recapitulating Tfam deletion.

P.12 BACH2 regulates thymic negative selection through restraining single-positive thymocyte activation

Alexander C. Evans, Alberto G. Conti, Yumi Yamashita-Kanemaru, Alexander J. Wesolowski, Charlotte J. Imianowksi, Benjamin I. Morris, Sarah K. Whiteside, Rahul Roychoudhuri, and Jie Yang

Department of Pathology, University of Cambridge, UK

T cell maturation and differentiation are critical for the development of a functional immune system. In the thymus, positive and negative selection of maturing thymocytes generates a polyclonal TCR repertoire and eliminates strongly self-reactive clones. The transcriptional repressor, BACH2, has been shown to play a critical role in regulating T lymphocyte differentiation, restraining terminal differentiation and function in peripheral tissues. However, its function within the thymic development of T cells remains unclear. Here, we investigated the role of BACH2 in regulating T cell maturation and differentiation during thymocyte development. Our findings demonstrate that BACH2 acts as an intrinsic negative regulator of T cell maturation and differentiation during the latter phases of selection. BACH2 is expressed in late-stage CD4+ CD8+ double-positive (DP) and single-positive (SP) thymocytes and supports SP thymocyte development by restricting activation in the presence of strong TCR stimulatory signals. Thymocyte subsets appear unperturbed in the absence of BACH2, however, the TCR repertoire of SP thymocytes is altered. Moreover, SP thymocytes lacking BACH2 display increased markers of activation, mimicking stronger affinity TCR-pMHC interactions, and resulting in greater apoptotic death from negative selection. These findings suggest that BACH2 plays a critical role in maintaining peripheral tolerance by regulating the maturation and differentiation of T cells during thymocyte development. Overall, this study highlights the precision control exerted by transcription factors during T cell development and their critical role in the maintenance of peripheral tolerance.

P.13 Giving Toll Like Receptor 4 Signalling an Angelic Glow

Gabrielle McClymont, Prassana Suresh, Joe Boyle, Clare Bryant

University of Cambridge, UK

Toll-like receptor 4 (TLR4) plays an essential role in the innate immune system protecting the body from gram negative bacteria. TLR4 has a both a protective and harmful inflammatory response, causing damage in conditions such as sepsis. Understanding the precise molecular mechanisms of the TLR4 signalling pathway is important to distinguishing between the protective and damaging TLR4 mediated immune responses and developing targeted therapies. This research focuses on understanding the kinetics, stoichiometry, cellular dynamics, and localisation of TIR adaptor protein Mal (also known as TIRAP) the first intracellular protein in the MyD88 dependent signalling pathway of TLR4. I have used CRISPR to create a macrophage cell line with Halo tagged Mal, allowing Mal to be visualised in individual macrophages. A variety of single cell approaches including live single molecule imaging and super resolution microscopy are utilised to collect physiologically relevant and accurate measurements of Mal during TLR4 stimulation with lipopolysaccharide (the primary ligand). Endogenously tagged cells have allowed us to overcome the over expression limitations of previous experimental designs. Preliminary results show are expanding our understanding of the role and localisation of Mal.

P.14 Exploring the synovial tissue-homing properties of human mononuclear phagocytes in active treatment naïve psoriatic arthritis.

Joseph Hutton[1,2,4], Deepak Jadon[2], Mariola Kurowska-Stolarska[3], Charlotte Summers[4], Naomi McGovern[1]

[1]Department of Pathology, University of Cambridge, UK [2]Rheumatology Research Unit, Cambridge University Hospitals NHS Foundation Trust, UK [3]School of Infection and Immunity, University of Glasgow, UK [4]Department of Medicine, University of Cambridge, UK

Psoriatic arthritis (PsA) is a common immune-mediated inflammatory disease with diverse manifestations including inflammatory arthritis, axial inflammation, enthesitis, dactylitis, nail disease, and extra-articular manifestations. The hypervascular synovium in PsA favours the delivery of circulating immune cells, creating a niche for infiltrating cells. Amongst the cells recruited to inflamed joints are mononuclear phagocytes (MPS) and osteoclasts. Within the joint, recruited cells adopt a pro-inflammatory profile contributing to tissue damage. The mechanisms that govern leukocyte recruitment across disease states and tissues are poorly understood. Using high-sequencing depth bulk RNA sequencing, single-cell RNA sequencing, and high-parameter flow and mass cytometry we sought to determine if circulating MPS have altered trafficking properties in PsA. We have found that that circulating MPS are enriched for transcripts that favour their adhesion, migration, and production of PsA-related cytokines, which may prime them for homing to the joint. Analysis of publicly-available datasets suggests that circulating classical monocytes likely differentiate into the synovial tissue macrophage subsets associated with disease activity. Using mass cytometry, we have found alterations in the expression of various migratory/adhesion markers and a putative circulating osteoclast precursor cell strongly enriched for synovial tissue homing signals. Ongoing work involves exploring the functional outcome of these differences between PsA and healthy controls.

P.15 Early infection response of the first-trimester placenta at single cell resolution

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The placenta functions as a selective barrier located at the interface between the mother and the fetus, supporting fetal nutrition and protection against pathological infections during pregnancy. However, some pathogens can interact and even cross the placenta, causing pregnancy complications which in some cases can have lifelong impacts on the child's health. Infections during pregnancy are a major burden worldwide but have been poorly studied owing to limited tissue availability. Here, we have optimised ex vivo first-trimester placental explants and generated the first single-cell census of placental cells upon infection with three pathogens associated with intrauterine complications - Plasmodium falciparum, Listeria monocytogenes and Toxoplasma gondii. We demonstrate that the trophoblasts, the specialised epithelial cells of the placenta, mount an inflammatory response, compromising placental function. Trophoblasts also upregulate chemokines that recruit both Hofbauer cells (fetal primitive macrophages in the placenta) and maternal macrophages (monocyte-derived macrophages attached to the placenta) to the site of infection. We show that independent of their origin, both fetal and maternal macrophages play an active role in the local inflammatory response. This reasearch shows for the first time that Hofbauer cells have an active role to combact infections in tissue.

P.16 Establishing tools to study the microbicidal capacity of human placental macrophages across gestation

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The placenta is a fetal-derived organ essential for a healthy pregnancy. It is made up of a complex network of villous trees, responsible for maternal-fetal exchange. Fetal-derived macrophages, termed Hofbauer cells (HBC), reside within the stroma of these structures. HBC are thought to play an important role in maintaining placental tissue homeostasis, development and defence against infection. However, our understanding of HBC lags far behind the rest of the macrophage field as equivalent cells are not found in the murine placenta. Hence, to develop our understanding of HBC, primary human placenta must be used. The McGovern lab have recently developed the tools to identify and isolate HBC from human placentas (Appios et al., 2021; Thomas et al., 2021). Through this study, we sought to develop protocols that would allow HBC to be maintained in vitro to study their interaction with transplacental pathogens. Through optimisation of our culture technique, we have found that HBC isolated from both first-trimester and term placentas can be maintained in vitro for at least 30 days. This is important as this is the only human tissue macrophage population that

can be maintained in vitro for a long period on a relatively large scale. Importantly, key differences between first-trimester and term HBC such as their HLA-DR expression status and key phagosomal properties are maintained in vitro. This has allowed us to study the differential interactions of pathogens with HBC isolated from both first-trimester and term placentas. Developing our understanding of host-pathogen interactions in the context of first-trimester and term HBC is critical for elucidating why certain pathogens present at different stages during pregnancy.

P.17 Multiviral Quartet Nanocages Elicit Broad Anti-Coronavirus Responses for Proactive Vaccinology

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Defending against future pandemics may require vaccine platforms that protect across a range of related pathogens. One solution that has been pioneered to address this problem is including multiple receptor-binding domains (RBDs) from evolutionarily-related viruses in a single mosaic vaccine. This approach involves stochastic presentation of several different sarbecovirus RBDs on a virus-like particle scaffold. Co-display of these antigens favours selection of B-cell receptors that bind evolutionarily conserved regions and consequently elicits a potent neutralizing response against these regions which are not the major target of conventional vaccines. Here we generated nanoparticles with dendritic architecture in which a quartet of tandemly-linked RBDs from human, bat and pangolin SARS-like betacoronaviruses (sarbecoviruses) couples on the mi3 nanocage through a SpyTag/SpyCatcher spontaneous reaction. These Quartet Nanocages induce a high level of antibodies against several different sarbecoviruses, including viruses not present in the vaccine, with responses exceeding the matched mosaic vaccine. The neutralizing antibodies raised against SARS-CoV, which is not represented in the vaccine, underline the potential for this strategy to confer heterotypic protection against emergent zoonotic coronavirus pathogens. In animals primed with SARS-CoV-2 Spike, boost immunizations with Quartet Nanocages increased the strength and breadth of an otherwise narrow immune response. The ability to generate a potent SARS-CoV-2 response with a Quartet immunogen lacking any SARS-CoV-2 sequence suggests the potential to generate pre-emptive vaccine libraries, where each member protects against a set of viruses, for proactive pandemic protection.

P.18 Investigating the roles of ZFP36 and ZFP36L1 RNA binding proteins in the regulation of CD8+ T cell functions

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CD8+ T cells target infected or malignant cells via direct cytotoxicity and the production of pro-inflammatory cytokines. Regulation of their function is required for effective yet restrained immune responses. The ZFP36 and ZFP36L1 RNA binding proteins contribute to the regulation of mRNA stability, protein translation and therein, CD8+ T cell function. The extent and significance of non-redundancy between ZFP36/L1 functions in CD8+ T cells, and the mechanisms that induce their expression, remain to be fully established. Here, we identify

stimuli differentially governing the expression of ZFP36/L1 and the roles of ZFP36/L1 in regulating pro-inflammatory cytokine production. We employ analysis of ZFP36/L1 expression in murine OTI memory-like T cells by western blot. Fusion reporter proteins, mAmetrineZFP36 and mCherryZFP36L1, are utilised to analyse ZFP36/L1 expression by flow cytometry. Flow cytometry was also applied to analyse cytokine production by conditional Zfp36 and/or Zfp36l1 KO memory-like T cells in response to PMA or ionomycin. We find that PMA stimulation strongly induces ZFP36 expression, while ZFP36L1 expression is greater in response to ionomycin than PMA stimulation alone. Despite ionomycin stimulation preferentially inducing both ZFP36L1 and IFN-γ expression, our results indicate that ZFP36 is the dominant repressor of both TNF-α production in response to PMA and IFN-γ production in response to ionomycin. Validation that fusion reporter models can be used to study ZFP36/L1 expression by flow cytometry enables further investigation of novel stimuli and signalling mechanisms regulating their expression. Additionally, this enables exploration of the relationship between antigen affinity and the expression of ZFP36/L1.

P.19 Effects of the commensal microbiota on T cell-mediated intestinal inflammation in response to immunotherapy.

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BACKGROUND: Immune-checkpoint inhibitors (ICI), cancer treatments that aid the immune system in recognising and attacking cancer cells, are utilised to treat a variety of cancers. However, colitis has been reported as a common side effect in more than 30% of patients who receive these treatments. In severe cases, patients are forced to suspend treatment. Increasing evidence has suggested a potential role of the intestinal microbiota in immune cell-mediated inflammation, including colitis, but the mechanisms in the context of ICI remain unclear.
METHODS: We established a mouse model using antibiotic-pre-treated anti-CTLA-4 monoclonal antibody (mAb) treated mice to monitor the dynamic change of immune cells and inflammatory markers in ICI treated animals. Flow cytometry was conducted to better characterise inflammatory gut T cell responses. 16S rRNA sequencing was conducted to explore gut microbial regulation of ICI-immunotherapy-induced intestinal inflammation. Gut inflammation was measured via Lipocalin 2 (Lcn-2) ELISA and histology.
RESULTS: We observed that anti-CTLA-4 mAb itself does not induce weight loss in mice; however, it does lead to decreased regulatory T cell (Treg) frequency and increased effector T cell (Teff) frequency in different compartments of the gut. Additionally, anti-CTLA-4 mAb treatment increased Lcn-2 and histological indications of inflammation in the small intestine in antibiotic-treated mice. Changes in microbial profiles were observed prior to inflammatory responses in a subset of mice.
CONCLUSIONS: Overall, these findings provide preliminary evidence that specific members of the microbiota correspond to T cell activation and intestinal inflammation in response to ICI immunotherapy. Further studies are required to provide a clearer picture of this specific intestinal inflammation mechanism and to support the development of potential therapies to alleviate side effects while maintaining cancer-treatment efficacy.

P.20 Identification of novel in vivo regulators of CD8 T cell persistence

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Correct balanced function of the immune system is required in the defence against infectious agents and transformed cells, however, overactive responses can lead to the development of autoimmune disorders or a failure to mount a response chronic infection or tumour progression. The tumour microenvironment is especially hostile towards effector immune cells such as CD8+ T cells whereby their function is impaired, and they become exhausted. While targeting surface exhaustion markers such as PD-1 results in clinical benefit to some patients there are others who do not respond, and the determining factors are fully understood. Here we sought to determine other novel regulators of CD8+ T cell exhaustion within the tumour microenvironment by performing in vivo CRISPR loss of function screens in immunocompetent mice. A bespoke library targeting genes that are differentially expressed in CD8+ T cells within the tumour microenvironment was screened. From this we have identified numerous genes that enabled enhanced survival or persistence of CD8+ T cells and confirmed the performance of our models by recovering known regulators such as Zc3h12a. Several of these genes have never previously been demonstrated have a functional role in CD8+ T cells and their mechanisms of action are currently under investigation.

P.21 Diminished Anti-HCMV T cell Functionality in the elderly may be ameliorated by inhibition of the PD-1: PD-L1 axis

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Human cytomegalovirus (HCMV) infection and periodic re-activation is, generally, well controlled by T-cell responses in the healthy. In ageing, while overt HCMV disease is not generally seen, HCMV infection is associated with increased risk of mortality and may be an important co-morbidity factor. HCMV genomes have been detected in the blood and bodily fluids of older people in a couple of studies, suggesting that whilst HCMV-specific immunity prevents HCMV-mediated disease, immunomodulation due to lifelong viral carriage may alter its efficacy. We have recently shown that there is a decrease in the efficacy of the adaptative immune response (total PBMC and T-cells sub-populations) to control viral infection and spread using a Viral Dissemination Assay in the elderly cohort. In addition, we have also demonstrated for the first time, a diminished HCMV neutralisation capacity of sera from the older donors. Phenotyping of fibroblasts generated by this study showed increased expression of checkpoint inhibitor ligands on the older donor derived fibroblasts which we hypothesise may contribute to the defect observed in cellular control of infection in the old. We have evidence that there is increased expression of inhibitory ligands HLA-E, B7-H3 and PD-L1 on fibroblasts from older donors. It is recognised that inhibitory PD-1 receptor is increased on T-cells from older adults. Therefore, utilising oncology blockade therapies already used in the clinic to target checkpoint pathway, may improve the T-cell effector response to HCMV

infection in the aged, we have preliminary evidence that disrupting the PD-1:PD-L1 axis improves control of HCMV dissemination.

P.22 Antigen drainage to the B cell follicle of the lymph node is impaired in aged mice

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Germinal centre (GC) response is the origin of high-affinity and long-lived humoral immunity. Defects of GC response are observed in ageing. It has been well established that initiation and magnitude of the GC response upon immunisation declines with age. However, the mechanism underlying it is not fully determined. Antigen access to the lymph node is the very beginning of the initiation of GC. Antigen flows to the lymph node via lymphatic vessels either freely or via carriage by dendritic cells. Previous work found that the velocity of the lymph draining towards the lymph node is reduced in aged rats, prompting the hypothesis that antigen drainage to the lymph node might be altered with age. To test this, we injected two sizes of nanoparticles, 20nm and 1000nm, that imitate antigen access either free or via dendritic cell mediated carriage correspondingly. We observed that drainage of 20nm nanoparticles to the B cell follicle of the murine lymph node is impaired in aged mice. However, there are no significant changes to larger 1000nm particles that are typically delivered by cells to the lymph node with age. As the B cell follicle is the place where GC develops, lack of access to antigen in the B cell follicle could be one of the factors that contribute to diminished GC response in ageing.

P.23 Building a description of the ex vivo immunometabolism of Leishmania panamensis-infected macrophages from RNA-seq data

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Macrophages are the main host cell of Leishmania parasites. The stimulus of the infection switches the basal immunometabolism of the macrophage to contain the infection. A holistic understanding of the immunometabolic network in response to intracellular parasites, has helped to pin down therapy and drug targets in different pathogen infections. Despite the prevalence of Leishmania. panamensis causing localized cutaneous leishmaniasis or more aggressive clinical manifestations, we do not know how the immunometabolism of the human host is reprogrammed during the infection. We use RNA-seq data to reconstruct the immunometabolic network displayed at 24 hours post-infection in L. panamensis-infected macrophages. We found evidence of an activated Hypoxia Transcription Factor potentially directing an early glycolytic flux. Alongside, negative regulators of glycolysis were upregulated. The redox metabolism was also regulated at the level of transcription by FOS, NOTCH3 and NFE2L2. The first two modulate the production of reactive oxygen species (ROS) while the last one contributes to the activation of a ROS scavenging system. Regarding the bioenergetic of the macrophage we found positively regulated components of oxidative phosphorylation, coupled with enzymes of the TCA cycle and fatty acid synthesis. On the immunological side, purinergic receptors might be directing inflammatory responses and adenosine receptors might be counteracting it. Despite the overexpression of receptors driving pro-inflammatory responses, we found some of the required downstream molecules either lacking, downregulated or negatively modulated by inhibitors. Overall, the immunometabolism in the

adaptation stage of L. panamensis-infected macrophages recalls a suitable modulatory profile for the survival of the parasite.

P.24 Development of a rapid screening platform for mRNA vaccines

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Vaccine development routinely requires testing in animal models as typical in vitro systems do not faithfully recapitulate the adaptive immune response seen in humans. However, in vivo screening of vaccine candidates is time consuming, labour intensive and often unproductive. To address this problem, we have developed an in vitro screening platform capable of rapidly assessing mRNA vaccine candidates for productive T and B cell antigen expression.
Using the SARS-CoV-2 RBD as an exemplar antigen we show that our system can identify candidate mRNA sequences that lead to altered antigen expression in an in vitro system that is recapitulated in an in vivo system. In particular, by altering codon usage within an mRNA sequence, expression of both T and B cell antigens can be increased in a cell culture system and also lead to stronger neutralising antibody responses when tested in vivo.
This in vitro system will allow us investigate potential strategies to induce stronger, antigen specific immune responses to vaccines for a variety of different antigens, such as by altering untranslated regions and codon usage within an mRNA sequence. This system also allows us to study unwanted immunogenicity and responses to mRNA based vaccines and therapies, including autoimmune diseases.

P.25 PI3Kδ activity in T or B cells: Different drivers of B cell lymphoma in the context of deregulated BCL6

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Signalling through the lipid-signalling kinase phosphoinositide 3-kinase δ (PI3Kδ) is essential for lymphocyte development and function. Hyperactivation of PI3Kδ induces Activated PI3Kδ Syndrome (APDS), a primary immunodeficiency characterised by recurrent respiratory infections and increased risk of B cell lymphoma.
To investigate the role PI3Kδ in B cell lymphoma, we crossed mice harbouring PI3Kδ hyperactivity to animals expressing deregulated B-cell Lymphoma 6 (µHABCL-6Tg), a common mouse model for Diffuse Large B Cell Lymphoma. To assess the effect of T and B cell-specific PI3Kδ activity on lymphomagenesis, we generated mouse models with germline (p110δE1020Kgl/WT µHABCL-6Tg), T cell-specific (CD4Cre p110δE1020Kflox/WT µHABCL-6Tg) and B cell-specific (Mb1Cre p110δE1020Kflox/WT µHABCL-6Tg) PI3Kδ activity on the µHABCL-6Tg background.

We used flow cytometry and Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-Seq) to phenotype lymphocyte populations in the generated mouse models. To demonstrate oncogenic transformation, we adoptively transferred B cells from the transgenic mice into immunodeficient (Rag2KO) hosts and assessed lymphomagenesis by histology and spectral flow cytometry.
Phenotyping revealed an increase in Germinal Centre (GC), Memory and Age-associated B cells in the germline and B cell-restricted mouse models. Adoptive transfer of B cells from all transgenic mouse models induced lymphoma after 75-100 days, characterised by changes in spleen morphology and weight. Furthermore, malignant B cells showed an increased expression of Memory and GC B cell markers.
This study highlights the capacity of PI3Kδ to induce malignancy through either T or B cells alone. Furthermore, it implicates a role for Memory B cells as potential drivers of lymphoma.

P.26 Integrin αvβ3 potentiates TH2 cell immunity

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T helper 2 (TH2) cell production of interleukin (IL)-13 drives asthma and allergic diseases. In response to IL4-induction, Gata3 is upregulated during TH2 cell differentiation and underlie anti-helminth immunity and misdirected allergic inflammation. Using an unbiased, whole mouse-genome CRISPR-Cas9 screens we identified a novel role for αvβ3 integrin in TH2 cell polarisation. Mechanistically, IL-4/Gata3-induced selective expression of αvβ3 permitted αvβ3-Thy1 intercellular interactions among TH2 cells, increased mTOR signalling, sustained differentiation and stimulated IL-5/IL-13 production. In murine models of allergic asthma, αvβ3 was required for optimal allergen-driven antigen-specific pulmonary adaptive type-2 responses. Similar to mice, human TH2 cells also differentially express αvβ3. Thus, via the expression of αvβ3, TH2 cells form multi-cellular factories to amplify type-2 responses.

P.27 Clustered invasion triggers cytosolic release of Salmonella Paratyphi A and subsequent cytosolic motility favors evasion of xenophagy

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Salmonella enterica is a common foodborne, facultative intracellular enteropathogen. Typhoidal S. enterica serovars like Paratyphi A (SPA) are human restricted and cause a severe systemic disease, while many S. enterica serovars like Typhimurium (STM) have broad host range, and in human hosts usually lead to self-limiting gastroenteritis. There are key differences between typhoidal and non-typhoidal Salmonella in pathogenesis, but underlying mechanisms remain largely unknown. Several genes encoding Salmonella pathogenicity island (SPI) effector proteins are absent or pseudogenes in SPA. Expression of virulence and metabolism genes show differential expression compared to STM. The intracellular transcriptomic architecture and phenotypes during presence in epithelial cells were recently described. Surprisingly, induction of motility, flagella and chemotaxis genes showed distinct expression patterns in intracellular SPA vs. STM and led to cytosolic motility of SPA. We applied

single cell microscopic analyses approaches to investigate the triggers and cellular consequences of cytosolic motility. Live cell imaging (LCI) revealed that SPA invades host cells in a highly cooperative manner. Extensive membrane ruffling at the invasion site leads to increased membrane damage in the nascent SCV with subsequent cytosolic release. After release into the cytosol, motile bacteria showed same velocity as under culture conditions used for infection. Reduced capture of SPA by autophagosomal membranes was observed by LCI and electron microscopy. Our results reveal flagella-mediated cytosolic motility as possible xenophagy evasion mechanism that could drive disease progression and contributes to dissemination of invasion-primed SPA during systemic infection.

P.28 CD14 marks tissue CD8$^+$T-cells instructed by myeloid cells and modulated by LPS

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Background and Aims: The liver is bathed in bacterial products, including lipopolysaccharide transported from the intestinal portal vasculature, but is able to maintain a state of tolerance that is then exploited by persistent pathogens and tumours. The cellular basis mediating this tolerance, yet allowing a switch to immunity or immunopathology, needs to be better understood for successful immunotherapy of liver diseases.
Method: We analysed the phenotype and function of CD14-expressing CD8 T cells directly *ex vivo* from resected/explanted human liver and explored their derivation, functionality, expansion and LPS-responsiveness in multiple *in vitro* and *in vivo* models.
Results: Here we show that a variable proportion of CD8$^+$ T cells compartmentalized in the human liver co-stain for CD14 and other prototypic myeloid membrane proteins and sit in close proximity to CD14^{high} myeloid cells in the liver. CD14$^+$CD8$^+$ T cells exhibit increased turnover, activation and constitutive immunomodulatory features with high homeostatic IL-10 and IL-2 production ex vivo, and enhanced antiviral/anti-tumour effector function after TCR engagement. Whereas stimulation via CD14 by bacterial lipopolysaccharide not only increases the frequency of CD14$^+$CD8$^+$ T cells in vitro and in vivo but skews their function towards the production of chemotactic and regenerative cytokines. This CD14$^+$CD8$^+$ T cell profile seen ex vivo in tissues, can be recapitulated by the acquisition of membrane proteins—including the

lipopolysaccharide receptor complex—from mononuclear phagocytes, resulting in augmented tumour killing by TCR-redirected T cells in vitro.
Conclusion: A proportion of CD8 T cells compartmentalised in the liver express CD14/TLR4/MD2, recapitulated *in vitro* by membrane acquisition from mononuclear phagocytes. Thus, bacterial products in the gut-liver axis and tissue stromal factors can fine tune liver immunity by driving myeloid instruction of $CD8^+$ T cells with immunomodulatory ability.